

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

Data Requirement:	PMRA DATA CODE {.....}
	EPA DP Barcode 431654
	OECD Data Point {.....}
	EPA MRID 49760108
	EPA Guideline 850.4500

Test material: Dithiopyr technical

Purity: 94.9%

Common name: Dithiopyr

Chemical name: IUPAC: Not reported

CAS name: 3,4-Pyridinecarbothioic acid, (2-difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-S-S-dimethyl ester

CAS number: Not reported

Synonyms: Not reported

Primary Reviewer: Dana Worcester
Environmental Scientist, CDM/CSS-Dynamac JV

Signature:

Date: 2/27/2017

Secondary Reviewer: John Marton, Ph.D.
Environmental Scientist, CDM/CSS-Dynamac JV

Signature:

Date: 3/09/2017

Primary Reviewer: Jennifer Connolly
GIS Biologist, EPA/OPP/EFED/EISB

Date: 11/2/17

JENNIFER
CONNOLLY

Digitally signed by
JENNIFER CONNOLLY
Date: 2018.06.13
19:01:46 -0400

Secondary Reviewer(s): Kristina Garber
Senior Science Advisor, EPA/OPP/EFED/ERB1

Date: 11/2/17

KRISTINA
GARBER

Digitally signed by
KRISTINA GARBER
Date: 2018.06.13
13:41:13 -0400

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel

Reference/Submission No.: {.....}

Company Code	{.....} [For PMRA]
Active Code	{.....} [For PMRA]
Use Site Category:	{.....} [For PMRA]
EPA PC Code	128994

Date Evaluation Completed: 11/17/17

CITATION: Arnie, J.R., L.A. Lockard, J.R. Porch and K.H. Martin. Dithiopyr TGAI: A 96-Hour Toxicity Test with the Marine Diatom (*Skeletonema costatum*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Project Number: 379P-114B. Study sponsored by Dow AgroSciences, LLC, Indianapolis, Indiana. Dow AgroSciences Study Number: 150426. Study initiated May 18, 2015 and completed October 16, 2015.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of marine diatom, *Skeletonema costatum*, were exposed to dithiopyr at nominal concentrations of 0 (negative control), 4.3, 9.4, 21, 45, and 100 µg/L under static conditions. No solvent was used in this study. The test substance declined substantially during the study, with 96-hour recoveries ranging from 49 to 54% of 0-hour concentrations. Therefore, the reviewer based toxicity values on initial measured concentrations which were <0.313 (<LOQ, negative control), 3.70, 8.46, 17.9, 38.3, and 84.1 µg ai/L.

After 96 hours, NOAEC values for yield and growth rate were 84.1 µg ai/L and for area under the growth curve was 38.3 µg ai/L. IC₅₀ values for yield, growth rate, and area under the growth curve were >84.1, >84.1, and 81.09 µg ai/L, respectively, in terms of initial measured concentrations. The % growth inhibition of cell density in the treated algal culture as compared to the negative control ranged from -9.8 to 42.3%.

No morphological abnormalities were noted. There were increases in pH during the test. There were no compound-related phytotoxic effects.

This study is scientifically sound and is classified as acceptable.

Results Synopsis

Test Organism: Marine diatom, *Skeletonema costatum* (strain not reported)

Test Type (Flow-through, Static, Static Renewal): Static

Note: N/A = not available

Yield

IC₀₅: 71.11 µg ai/L 95% C.I.: 0 to 81.93 µg ai/L

IC₅₀: >84.1 µg ai/L 95% C.I.: N/A

NOAEC: 38.3 µg ai/L

Probit Slope: N/A

Growth rate

IC₀₅: Not calculable 95% C.I.: N/A

IC₅₀: >84.1 µg ai/L 95% C.I.: N/A

NOAEC: 84.1 µg ai/L

Probit Slope: N/A

Area under the curve

IC₀₅: 68.57 µg ai/L 95% C.I.: 0 to 72.08 µg ai/L

IC₅₀: 81.09 µg ai/L 95% C.I.: 79.85 to 82.34 µg ai/L

NOAEC: 38.3 µg ai/L

Probit Slope: N/A

Endpoint(s) Effected: Yield and area under the curve.

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was performed according to the procedures of OCSPP 850.4500 (2012), OECD Guideline 201 (2006), and the Official Journal of the European Communities No. L383, Method C.3 (1992). The following deviations from the U.S. EPA OCSPP 850.4500 (2012) guideline are noted:

1. The strain of the test organism was not reported.
2. The algae were maintained under continuous lighting. OCSPP guidance requires *Skeletonema costatum* to be maintained under a 14-hour light:10 dark schedule.
3. Test concentrations declined substantially during the study, with 96-hour measured concentrations ranging from 49 to 54% of their initial measured counterparts.
4. The pH, TOC, particulate matter, and metals, and chlorine concentrations of the dilution water were not reported.
5. The physico-chemical properties of the test material were not reported.

These deviations do not impact the acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study was performed in accordance with the GLP standards of U.S. EPA (40 CFR Parts 160 and 792), OECD GLP, and Japan MAFF with the following exception: periodic analyses of well water for potential contaminants were not performed according to Good Laboratory Standards, but were performed using a certified laboratory and standard US EPA analytical methods.

A. MATERIALS:

1. Test material Dithiopyr technical

Description: Solid

Lot No./Batch No. : 1A08164B01

Purity: 94.9%

Stability of compound under test conditions: The 96-hour measured concentrations ranged from 49 to 54% of their initial measured counterparts. See reviewer comments.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Ambient temperature in the dark.

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

Physicochemical properties of Dithiopyr.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Name: Marine diatom, *Skeletonema costatum*

*EPA requires a nonvascular species: For tier I testing, only one species, *S. capricornutum*, to be tested; for tier II testing, *S. costatum*, *A. flos-aquae*, *S. capricorntum*, and a freshwater diatom is tested.*

*OECD suggests the following species are considered suitable: *S. capricornutum*, *S. subspicatus*, and *C. vulgaris*. If other species are used, the strain should be reported*

Strain: Not reported

Source: In-house cultures originally obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP).

Age of inoculum: 3 days

Method of cultivation: Cultured in Enriched Saltwater Media under continuous fluorescent light at 4300 ±10% lux at 20 ± 2°C and shaken at 100 rpm.

B. STUDY DESIGN:

1. Experimental Conditions

- a. Range-finding study: An exploratory, non-GLP, range-finding toxicity test was conducted at nominal concentrations of 0.10, 1.0, 10 and 100 µg ai/L, and yielded -17, -19, -27, and 98% inhibition of mean cell density, respectively, after 96 hours of exposure, relative to the mean negative control response.
- a. b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	Continuously cultured in-house	
Culturing media and conditions:	Same as test	

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

Parameter	Details	Remarks
		Criteria
(same as test or not)		<i>EPA recommends two week acclimation period.</i>
Health: (any mortality observed)	Actively growing for at least two weeks.	<i>OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.</i>
Test system Static/static renewal	Static	
Renewal rate for static renewal	N/A	<i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i>
Incubation facility	Temperature-controlled environmental chamber with a shaker table	
Duration of the test	96 hours	<i>EPA requires: 96-120 hours OECD: 72 hours</i>
Test vessel Material: (glass/stainless steel) Size: Fill volume:	Glass 250 mL 100 mL	Erlenmeyer plugged with sterile foam stoppers. <i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i>
Details of Enriched saltwater		

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

Parameter	Details	Remarks
		Criteria
medium pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	8.0 8.4 to 8.8 Na ₂ EDTA·2H ₂ O N/A Approximately 30 ppt	<i>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</i> <i>EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).</i>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	
Dilution water source/type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Purified (NANOpure water) Not reported N/A Sterilized and filtered Not reported Not reported Not reported Below detection limits Not reported	<i>EPA pH: <i>Skeletonema costatum</i> = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.</i> <i>OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.</i>

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

Parameter	Details	Remarks
		Criteria
Indicate how the test material is added to the medium (added directly or used stock solution)	A primary stock solution was prepared by dissolving 0.0105 g of the test substance in 100 mL acetonitrile to achieve a nominal concentration of 100 µg ai/mL. Four additional stock solutions were prepared by adding 500 µL of each stock to a 500 mL glass volumetric flask, evaporating the solvent under a stream of nitrogen. After the solvent evaporated, saltwater algal medium was added.	
Aeration or agitation	Constantly shaken at rate of 100 rpm	
Initial cells density	10,000 cells/mL	<p><i>EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.</i></p> <p><i>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</i></p>

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

Parameter	Details	Remarks
		Criteria
Number of replicates Control: Solvent control: Treatments:	8 N/A 4	<p>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula sp.</i> tests should be conducted with four replicate.</p> <p>OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.</p>
Test concentrations Nominal: Geometric mean measured: Initial measured:	0 (negative control), 4.3, 9.4, 21, 45, and 100 µg/L <0.313 (<LOQ, negative control), 2.6, 5.9, 13, 28, and 61 µg ai/L <0.313 (<LOQ, negative control), 3.70, 8.46, 17.9, 38.3, and 84.1 µg ai/L	<p>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</p> <p>OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.</p>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	Test concentrations were measured at 0 and 96 hours using HPLC analysis.	

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

Parameter	Details	Remarks
		Criteria
Test conditions Temperature: Photoperiod: Light intensity and quality:	19.29 to 19.85°C Continuous 4060-4680 lux fluorescent light	<p>EPA temperature: <i>Skeletonema</i>: 20EC, Others: 24-25EC; EPA photoperiod: <i>S. costatum</i> 14 hr light/ 10 hr dark, Others: Continuous; EPA light: <i>Anabaena</i>: 2.0 Klux ($\pm 15\%$), Others: 4 - 5 Klux ($\pm 15\%$)</p> <p>OECD recommended the temperature in the range of 21 to 25°C maintained at $\pm 2^\circ\text{C}$ and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.</p>
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks
		Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density Yield Growth rate Area under the curve (AUC)	EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

Parameters	Details	Remarks
		Criteria
Measurement technique for cell density and other end points	Cell density was determined using a light microscope and hemocytometer (Improved Neubauer). Yield was calculated as cell density at test termination minus test initiation. Growth rate was calculated from cell density using a logarithmic growth equation. AUC was calculated as the area under the cell density growth curve.	<p><i>EPA recommends the measurement technique of cell counts or chlorophyll a</i></p> <p><i>OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).</i></p>
Observation intervals	Every 24 hours	<i>EPA and OECD: every 24 hours.</i>
Other observations, if any	Cells were observed daily for health.	
Indicate whether there was an exponential growth in the control	Yes, after 96 hours, the mean cell density of the negative control increased 100-fold.	<p><i>EPA requires control cell count at termination to be □ 2X initial count or by a factor of at least 16 during the test.</i></p> <p><i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i></p>
Were raw data included?	Yes, raw cell density data were provided.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 96 hours, the mean cell density of the negative control was 172.88×10^4 cells/mL, yielding inhibitions relative to the negative control of -9.8, -6.6, -4.9, -5.0 and 42.3% for initial measured exposure concentrations of 3.70, 8.46, 17.9, 383, and 84.1 µg ai/L, respectively. The study author did not assess cell density.

The mean 0-96 hour yield of the negative control was 171.88×10^4 cells/mL, yielding inhibitions relative to the negative control of -9.9, -6.6, -4.9, -5.1, and 42.5% for the initial measured exposure concentrations of 3.70, 8.46, 17.9, 383, and 84.1 µg ai/L, respectively. The study author reported inhibitions of -10, -7, -5, -5 and 43% for the geometric mean measured exposure concentrations of 2.6, 5.9, 13, 28, and 61 µg ai/L, respectively. The NOAEC and IC₅₀ values reported by the study author based on yield were 28 and >61 µg ai/L, respectively, in terms of geometric mean measured exposure concentrations.

The mean 0-96 hour growth rate of the negative control was 1.287/day, yielding inhibitions relative to the negative control of -1.8, -1.3, -1.0, -0.9, and 21.0% for the initial measured exposure concentrations of 3.70, 8.46, 17.9, 383, and 84.1 µg ai/L, respectively. The study author reported inhibitions of -2, -1, -1, -1 and 21% for the

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

geometric mean measured exposure concentrations of 2.6, 5.9, 13, 28, and 61 µg ai/L, respectively. The NOAEC and IC₅₀ values reported by the study author based on growth rate were 28 and >61 µg ai/L, respectively, in terms of geometric mean measured exposure concentrations.

The mean 0-96 hour AUC value of the negative control was 5327, yielding inhibitions relative to the negative control of -5.5, -3.6, -10.8, -8.5, and 62.3% for the initial measured exposure concentrations of 3.70, 8.46, 17.9, 383, and 84.1 µg ai/L, respectively. The study author reported inhibitions of -6, -4, -11, -9 and 62% for the geometric mean measured exposure concentrations of 2.6, 5.9, 13, 28, and 61 µg ai/L, respectively. The NOAEC and IC₅₀ values reported by the study author based on AUC were 28 and 54 µg ai/L, respectively, in terms of mean measured exposure concentrations.

No morphological abnormalities were noted. There were increases in pH during the test. There were no compound-related phytotoxic effects.

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

Table 3: Measured concentrations of Dithiopyr (marine diatom, *Skeletonema costatum*)

Treatment Initial measured (geometric mean measured) and [nominal] concentrations µg ai/L	Measured Concentrations	
	0 hours (Day 0)	96 hours (Day 4)
Negative control	<LOQ	0.0451
3.70 [4.3]	3.70	1.83
8.46 [9.4]	8.46	4.13
17.9 [21]	17.9	9.25
38.3 [45]	38.3	20.5
84.1 [100]	84.1	44.3

Table 4: Effect of Dithiopyr on algal growth (marine diatom, *Skeletonema costatum*)

Treatment Initial measured and [nominal] concentrations µg ai/L	Initial cell density (x10 ⁴ cells/mL)	Cell density (x10 ⁴ cells/mL) at			
		48 hours	72 hours	96 hours	
				cell count	% inhibition ^a
Negative control	1.0	22.45	112.16	172.88	N/A
3.70 [4.3]	1.0	22.40	115.35	189.88	-9.8
8.46 [9.4]	1.0	23.53	113.35	184.25	-6.6
17.9 [21]	1.0	23.15	129.28	181.35	-4.9
38.3 [45]	1.0	21.78	125.8	181.58	-5
84.1 [100]	1.0	9.38	24.9	99.83	42.3
Reference chemical (if used)	N/A				

^a Calculated by the reviewer relative to the negative control.

Table 5: Effect of Dithiopyr algal growth (marine diatom, *Skeletonema costatum*)

Treatment Initial measured and [nominal] concentrations µg ai/L	Initial cell density (x10 ⁴ cells/mL)	Mean growth rate (day ⁻¹)		Mean area under the curve (AUC)		Mean yield (based on cell density; x10 ⁴ cells/mL)	
		0-96 hours	% inhibition ^a	0-96 hours	% inhibition ^a	0-96 hours	% inhibition ^a
Negative control	1.0	1.287	N/A	5327	N/A	171.88	N/A
3.70 [4.3]	1.0	1.311	-1.8	5619	-5.5	188.88	-9.9
8.46 [9.4]	1.0	1.304	-1.3	5518	-3.6	183.25	-6.6

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

17.9 [21]	1.0	1.300	-1.0	5902	-10.8	180.35	-4.9
38.3 [45]	1.0	1.300	-0.9	5780	-8.5	180.58	-5.1
84.1 [100]	1.0	1.017	21.0	2010	62.3	98.83	42.5

^a Calculated by the reviewer relative to the negative control.

Table 6: Statistical endpoint values.* (calculated by the study author based on geometric mean measured concentrations)

Statistical endpoint	Cell density	Yield	Growth rate	Area under the curve (AUC)
NOAEC ($\mu\text{g ai/L}$)	Not calculated	28	28	28
LOAEC ($\mu\text{g ai/L}$)	Not calculated	Not calculated	Not calculated	Not calculated
IC ₀₅ or EC ₀₅ ($\mu\text{g ai/L}$) (95% C.I.)	Not calculated	Not calculated	Not calculated	Not calculated
IC ₂₀ or EC ₂₀ ($\mu\text{g ai/L}$) (95% C.I.)	Not calculated	Not calculated	Not calculated	Not calculated
IC ₅₀ or EC ₅₀ ($\mu\text{g ai/L}$) (95% C.I.)	Not calculated	>61 (N/A)	>61 (N/A)	54 (43 to 61)
Reference chemical, if used	N/A			

* Do not use this table, if the study was deemed unacceptable.

NA = Not applicable.

B. REPORTED STATISTICS:

The study author statistically analyzed the endpoints for mean growth rates and yield using The SAS System for Windows Version 9.4. EC₅₀ values were calculated using nonlinear regression. The Shabenberger Hormetic Logistic model was used to estimate EC₅₀ values and their corresponding 95% confidence intervals as this model was a better fit for the data when compared to other non-linear regression models. The data were assessed for normality and homogeneity of variance using Shapiro-Wilk's and Levene's tests, respectively. If the data passed these tests, Dunnett's test was used to determine the NOAEC. If the data did not pass, the NOEC was determined using Jonckheere-Terpstra Step-Down Trent test. All analyses were based on geometric mean measured concentrations.

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer statistically analyzed the 96-hour endpoints for yield, growth rate, and AUC using CETIS version 1.8.7.12 statistical software using backend database settings implemented by EFED on 10/20/15. The data were assessed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's tests, respectively. Growth rate and yield data had non-normal distribution and unequal variances and were therefore analyzed using the Mann-Whitney test. AUC data had normal distribution and unequal variances and was analyzed using Mann-Whitney. The IC_x values were calculated using Bruce-Versteeg regression. Toxicity values are reported in terms of initial measured exposure concentrations.

Yield

IC ₀₅ :	71.11 µg ai/L	95% C.I.: N/A to 81.93 µg ai/L
IC ₅₀ :	>84.1 µg ai/L	95% C.I.: N/A
NOAEC:	38.3 µg ai/L	
Probit Slope:	N/A	

Growth rate

IC ₀₅ :	Not calculable	95% C.I.: N/A
IC ₅₀ :	>84.1 µg ai/L	95% C.I.: N/A
NOAEC:	84.1 µg ai/L	
Probit Slope:	N/A	

Area under the curve

IC ₀₅ :	68.57 µg ai/L	95% C.I.: N/A to 72.08 µg ai/L
IC ₅₀ :	81.09 µg ai/L	95% C.I.: 79.85 to 82.34 µg ai/L
NOAEC:	38.3 µg ai/L	
Probit Slope:	N/A	

D. STUDY DEFICIENCIES:

1. The strain of the test organism was not reported.
2. The algae were maintained under continuous lighting. OCSPP guidance requires *Skeletonema costatum* to be maintained under a 14-hour light:10 dark schedule.
3. The test substance was unstable under test conditions for the test solutions, with 96-hour measured concentrations ranging from 49 to 54% of their initial measured counterparts.
4. The pH, TOC, particulate matter, and metals, and chlorine concentrations of the dilution water were not reported.
5. The physico-chemical properties of the test material were not reported.

E. REVIEWER'S COMMENTS:

The reviewer's IC₅₀ values were in general agreement with those reported by the study author. The reviewer's NOAEC value was in agreement with the study author's NOAEC value for area under the growth curve (AUC), but differed for yield and growth rate. The reviewer determined a NOAEC value of 84.1 µg ai/L for these endpoints, based on initial measured concentrations, whereas the study author determined a NOAEC value of 28 µg ai/L, based on geometric mean measured concentrations. The reviewer's results are reported in the Executive Summary and Conclusions sections of this report.

As part of the stock solution preparation, the test material was dissolved in acetonitrile, which was evaporated off. Saltwater algal medium was added to complete the stock solutions. The study authors indicated that this procedure was carried out to increase accuracy associated with test concentrations and in order to avoid the use

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

of a solvent in the test. Similar approaches have been attempted in sediment toxicity studies with invertebrates. There is uncertainty associated with the addition of acetonitrile to the stock solutions, as this was not incorporated into the controls. This approach assumes that acetonitrile is completely evaporated prior to addition of the saltwater medium and so algae are not exposed to acetonitrile.

Dithiopyr was detected on day 4 at 0.0451 ug a.i./L in the control. Since the validity criteria were met, and the concentration was 2-3 orders of magnitude below the test levels, it was assumed that this low level of contamination did not impact the validity of the study.

Since this compound has a Koc ranging 1396-3049, and the chemical is stable due to hydrolysis, photolysis half-lives are on the order of weeks and aerobic soil metabolism half lives are >1 year (based on information in the registration review problem formulation), it is the reviewer's opinion that the lack of stability of the compound in the test vessels is due to sorption, not degradation.

The in-life phase of the definitive test was conducted from 15 to 19 June 2015.

The coefficient of variation (CV) of control yield was 7.87%, which meets the guideline requirement of yield CV<15%. The CV of control growth rate was 1.54%, which meets the guideline requirement of growth rate CV<15%.

F. CONCLUSIONS:

This study is **scientifically sound** and is classified as **acceptable**. After 96 hours, the most sensitive endpoint was area under the curve with NOAEC and IC₅₀ values of 38.3 and 81.09 µg ai/L, respectively, based on initial measured concentrations.

Yield

IC ₀₅ :	71.11 µg ai/L	95% C.I.: N/A to 81.93 µg ai/L
IC ₅₀ :	>84.1 µg ai/L	95% C.I.: N/A
NOAEC:	38.3 µg ai/L	
Probit Slope:	N/A	

Growth rate

IC ₀₅ :	Not calculable	95% C.I.: N/A
IC ₅₀ :	>84.1 µg ai/L	95% C.I.: N/A
NOAEC:	84.1 µg ai/L	
Probit Slope:	N/A	

Area under the curve

IC ₀₅ :	68.57 µg ai/L	95% C.I.: N/A to 72.08 µg ai/L
IC ₅₀ :	81.09 µg ai/L	95% C.I.: 79.85 to 82.34 µg ai/L
NOAEC:	38.3 µg ai/L	
Probit Slope:	N/A	

Endpoint(s) Effected: Yield and area under the curve.

III. REFERENCES:

Organization for Economic Cooperation and Development. 2006. OECD Guidelines for Testing of Chemicals, Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. Adopted 23 March 2006.

Data Evaluation Report on the Acute Toxicity of Dithiopyr to Algae, *Skeletonema costatum*

PMRA Submission Number {.....}

EPA MRID Number 49760108

Official Journal of the European Communities. 1992. No. L383. Method C.3. Algal Inhibition Test.

U.S. Environmental Protection Agency. 2012. Series 850-Ecological Effects Test Guidelines, OCSPP Number 850.4500: Algal Toxicity.

American Society for Testing and Materials. 2004. ASTM Standard Guide E1218-04. Standard Guide for Conducting Static Toxicity Tests with Microalgae.

The SAS System for Windows. 2002-2014. Version 9.4. SAS Institute, Inc., Gary, North Carolina.

Schabenberger et. al. 1999. Statistical Tests for Hormesis and Effective Dosages in Herbicide Dose Response. Agronomy Journal. 91: 713-721.

CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 1 of 6)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity								Wildlife International										
Analysis ID:		12-1582-1418	Endpoint:			96h AUC	CETIS Version:		CETISv1.8.7									
Analyzed:		23 Mar-17 19:57	Analysis:			Nonparametric-Two Sample	Official Results:		Yes									
Batch ID:	14-4880-3315	Test Type:			Algal Cell Growth (96-h)			Analyst:										
Start Date:	18 May-15	Protocol:			OCSPP 850.4500 Aquatic Plant (Algae)			Diluent:										
Ending Date:		Species:			Skeletonema costatum			Brine:										
Duration:	NA	Source:			Center for Culture of Marine Phytoplankton,			Age:										
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU									
Untransformed	NA	C > T	NA	NA	13.0%	38.3	84.1	56.75										
Mann-Whitney U Two-Sample Test																		
Control	vs	C-µg ai/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α :5%)									
Negative Control	3.7	9	NA	0	10	0.8929	Exact	Non-Significant Effect										
	8.46	13	NA	0	10	0.7152	Exact	Non-Significant Effect										
	17.9	1	NA	0	10	0.9980	Exact	Non-Significant Effect										
	38.3	4	NA	0	10	0.9859	Exact	Non-Significant Effect										
	84.1*	32	NA	0	10	0.0020	Exact	Significant Effect										
ANOVA Table																		
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α :5%)										
Between	44776130		8955227		5	22.98	<0.0001	Significant Effect										
Error	8571865		389630.2		22													
Total	53348000				27													
Distributional Tests																		
Attribute	Test		Test Stat	Critical	P-Value	Decision(α :1%)												
Variances	Bartlett Equality of Variance		20.6	15.09	0.0010	Unequal Variances												
Distribution	Shapiro-Wilk W Normality		0.9327	0.8975	0.0721	Normal Distribution												
96h AUC Summary																		
C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect							
0	Negative Control	8	5327	4989	5664	5198	4855	5866	142.7	7.58%	0.0%							
3.7		4	5619	5059	6179	5728	5137	5885	175.9	6.26%	-5.5%							
8.46		4	5518	5039	5997	5588	5098	5797	150.5	5.46%	-3.59%							
17.9		4	5902	5745	6058	5882	5804	6037	49.1	1.66%	-10.8%							
38.3		4	5780	5185	6374	5862	5261	6133	186.9	6.47%	-8.51%							
84.1		4	2010	-303.5	4323	2119	318	3482	726.9	72.33%	62.27%							
Graphics																		

CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 2 of 6)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity							Wildlife International					
Analysis ID:		11-8417-1219	Endpoint:			96h AUC	CETIS Version:		CETISv1.8.7			
Analyzed:		23 Mar-17 19:57	Analysis:			Nonparametric-Control vs Ord. Treatments	Official Results:		Yes			
Batch ID:	14-4880-3315	Test Type:			Algal Cell Growth (96-h)			Analyst:				
Start Date:	18 May-15	Protocol:			OCSPP 850.4500 Aquatic Plant (Algae)			Diluent:				
Ending Date:		Species:			Skeletonema costatum			Brine:				
Duration:	NA	Source:			Center for Culture of Marine Phytoplankton,			Age:				
Data Transform	Zeta	Alt Hyp	Trials	Seed			NOEL	LOEL	TOEL	TU		
Untransformed	NA	C > T	NA	NA			84.1	>84.1	NA			
Jonckheere-Terpstra Step-Down Test												
Control	vs	C- μ g ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)			
Negative Control		3.7	9	NA	-2	0.9970	Exact	Non-Significant Effect				
		8.46	33	NA	-2	0.9970	Exact	Non-Significant Effect				
		17.9	38	NA	-2	0.9970	Exact	Non-Significant Effect				
		38.3	60	NA	-2	0.9970	Exact	Non-Significant Effect				
		84.1	156	NA	-2	0.5714	Exact	Non-Significant Effect				
ANOVA Table												
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision(α :5%)			
Between	44776130		8955227		5	22.98		<0.0001	Significant Effect			
Error	8571865		389630.2		22							
Total	53348000				27							
Distributional Tests												
Attribute	Test		Test Stat	Critical	P-Value	Decision(α :1%)						
Variances	Bartlett Equality of Variance		20.6	15.09	0.0010	Unequal Variances						
Distribution	Shapiro-Wilk W Normality		0.9327	0.8975	0.0721	Normal Distribution						
96h AUC Summary												
C- μ g ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Negative Control	8	5327	4989	5664	5198	4855	5866	142.7	7.58%	0.0%	
3.7		4	5619	5059	5728	5137	5885	175.9	6.26%	-5.5%		
8.46		4	5518	5039	5997	5588	5098	5797	150.5	5.46%	-3.59%	
17.9		4	5902	5745	6058	5882	5804	6037	49.1	1.66%	-10.8%	
38.3		4	5780	5185	6374	5862	5261	6133	186.9	6.47%	-8.51%	
84.1		4	2010	-303.5	4323	2119	318	3482	726.9	72.33%	62.27%	
Graphics												

CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 3 of 6)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	17-7390-3534	Endpoint:	96h Cell Density	CETIS Version:	CETISv1.8.7				
Analyzed:	23 Mar-17 19:57	Analysis:	Nonparametric-Two Sample	Official Results:	Yes				
Batch ID:	14-4880-3315	Test Type:	Algal Cell Growth (96-h)	Analyst:					
Start Date:	18 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media				
Ending Date:		Species:	Skeletonema costatum	Brine:					
Duration:	NA	Source:	Center for Culture of Marine Phytoplankton,	Age:					
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	21.0%	84.1	>84.1	NA	

Mann-Whitney U Two-Sample Test

Control	vs	C-µg ai/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		3.7	6	NA	0	10	0.9596	Exact	Non-Significant Effect
		8.46	8	NA	0	10	0.9172	Exact	Non-Significant Effect
		17.9	9	NA	0	10	0.8848	Exact	Non-Significant Effect
		38.3	12	NA	0	10	0.7576	Exact	Non-Significant Effect
		84.1	24	NA	0	10	0.1010	Exact	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	23176.69	4635.338	5	4.381	0.0064	Significant Effect
Error	23274.76	1057.943	22			
Total	46451.45		27			

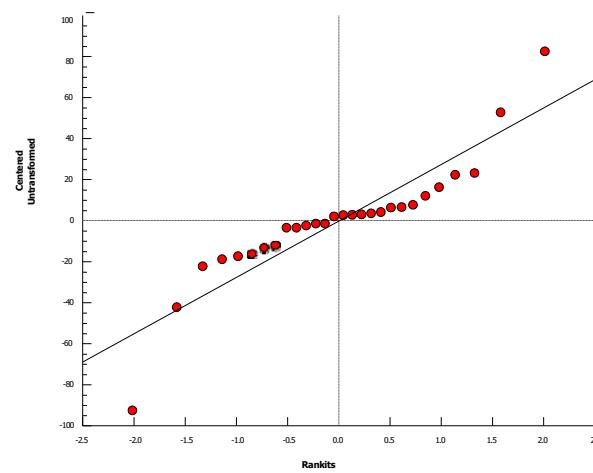
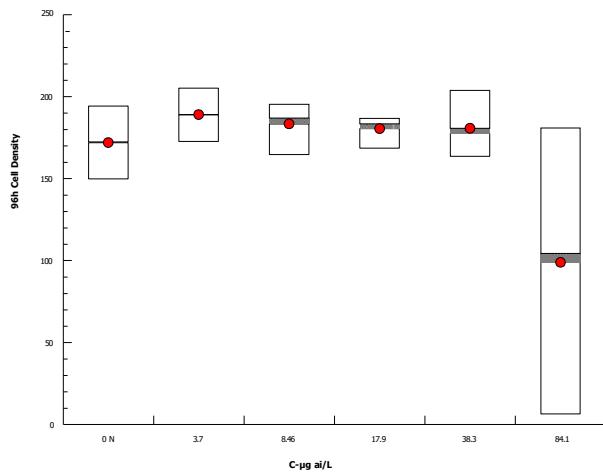
Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	26.46	15.09	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8641	0.8975	0.0018	Non-normal Distribution

96h Cell Density Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	171.9	160.6	183.2	172.1	149.6	194.2	4.783	7.87%	0.0%
3.7		4	188.9	167.2	210.6	188.9	172.5	205.2	6.826	7.23%	-9.89%
8.46		4	183.2	162.2	204.3	186.6	164.4	195.3	6.613	7.22%	-6.62%
17.9		4	180.4	167.3	193.4	183.2	168.3	186.7	4.101	4.55%	-4.93%
38.3		4	180.6	153.6	207.5	177.6	163.2	203.8	8.461	9.37%	-5.06%
84.1		4	98.83	-30.61	228.3	104.1	6.3	180.8	40.67	82.31%	42.5%

Graphics



CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 4 of 6)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	10-2424-2738	Endpoint:	96h Cell Density	CETIS Version:	CETISv1.8.7
Analyzed:	23 Mar-17 19:57	Analysis:	Nonparametric-Control vs Ord. Treatments	Official Results:	Yes
Batch ID:	14-4880-3315	Test Type:	Algal Cell Growth (96-h)	Analyst:	
Start Date:	18 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media
Ending Date:		Species:	Skeletonema costatum	Brine:	
Duration:	NA	Source:	Center for Culture of Marine Phytoplankton,	Age:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	84.1	>84.1	NA	

Jonckheere-Terpstra Step-Down Test

Control	vs	C-µg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		3.7	6	NA	-2	0.9636	Exact	Non-Significant Effect	
		8.46	23	NA	-2	0.9580	Exact	Non-Significant Effect	
		17.9	55	NA	-2	0.8818	Exact	Non-Significant Effect	
		38.3	98	NA	-2	0.7688	Exact	Non-Significant Effect	
		84.1	180	NA	-2	0.2175	Exact	Non-Significant Effect	

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	23176.69	4635.338	5	4.381	0.0064	Significant Effect
Error	23274.76	1057.943	22			
Total	46451.45		27			

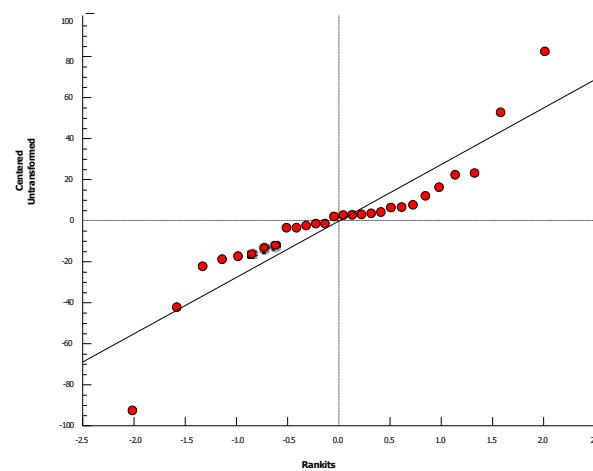
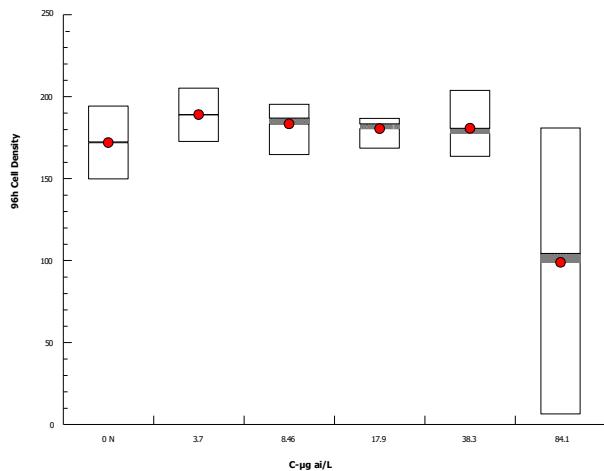
Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	26.46	15.09	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8641	0.8975	0.0018	Non-normal Distribution

96h Cell Density Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	171.9	160.6	183.2	172.1	149.6	194.2	4.783	7.87%	0.0%
3.7		4	188.9	167.2	210.6	188.9	172.5	205.2	6.826	7.23%	-9.89%
8.46		4	183.2	162.2	204.3	186.6	164.4	195.3	6.613	7.22%	-6.62%
17.9		4	180.4	167.3	193.4	183.2	168.3	186.7	4.101	4.55%	-4.93%
38.3		4	180.6	153.6	207.5	177.6	163.2	203.8	8.461	9.37%	-5.06%
84.1		4	98.83	-30.61	228.3	104.1	6.3	180.8	40.67	82.31%	42.5%

Graphics



OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	05-3046-2470	Endpoint:	96h Growth Rate	CETIS Version:	CETISv1.8.7
Analyzed:	23 Mar-17 19:57	Analysis:	Nonparametric-Two Sample	Official Results:	Yes
Batch ID:	14-4880-3315	Test Type:	Algal Cell Growth (96-h)	Analyst:	
Start Date:	18 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media
Ending Date:		Species:	Skeletonema costatum	Brine:	
Duration:	NA	Source:	Center for Culture of Marine Phytoplankton,	Age:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	11.8%	84.1	>84.1	NA	

Mann-Whitney U Two-Sample Test

Control	vs	C-µg ai/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		3.7	6	NA	0	10	0.9596	Exact	Non-Significant Effect
		8.46	8	NA	0	10	0.9172	Exact	Non-Significant Effect
		17.9	9	NA	0	10	0.8869	Exact	Non-Significant Effect
		38.3	11.5	NA	1	10	0.7758	Exact	Non-Significant Effect
		84.1	24	NA	0	10	0.1010	Exact	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2729224	0.05458447	5	2.891	0.0374	Significant Effect
Error	0.4154215	0.0188828	22			
Total	0.6883439		27			

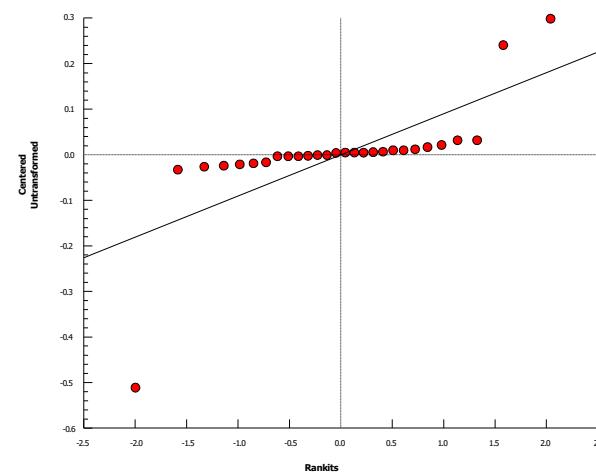
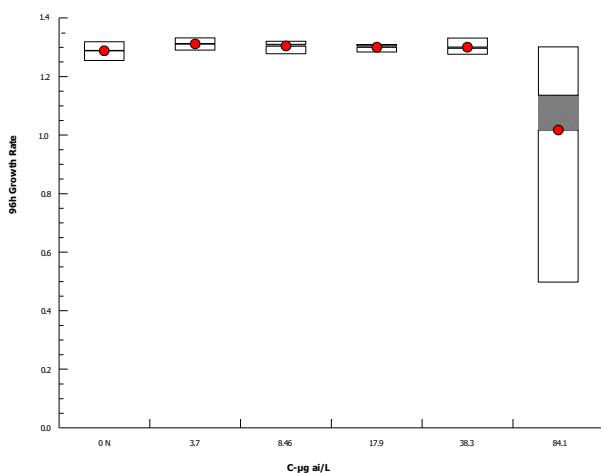
Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	63.49	15.09	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.5428	0.8975	<0.0001	Non-normal Distribution

96h Growth Rate Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	1.288	1.271	1.304	1.289	1.254	1.319	0.006987	1.54%	0.0%
3.7		4	1.311	1.282	1.34	1.312	1.289	1.332	0.008972	1.37%	-1.83%
8.46		4	1.304	1.274	1.333	1.309	1.277	1.32	0.009295	1.43%	-1.26%
17.9		4	1.3	1.282	1.318	1.304	1.283	1.309	0.005788	0.89%	-0.97%
38.3		4	1.3	1.263	1.337	1.296	1.275	1.331	0.01159	1.78%	-0.95%
84.1		4	1.017	0.4297	1.604	1.135	0.497	1.301	0.1845	36.29%	21.01%

Graphics

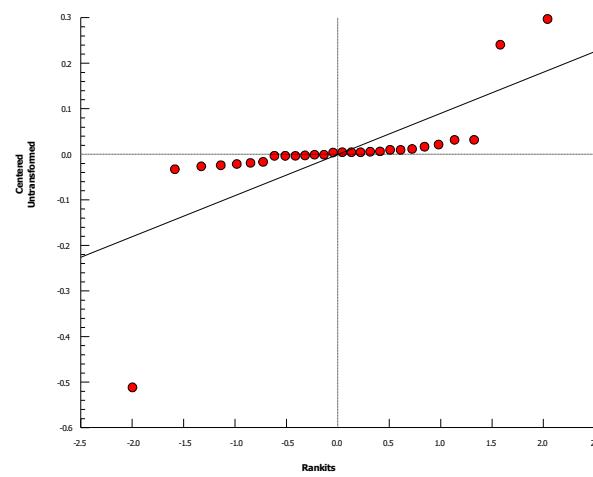
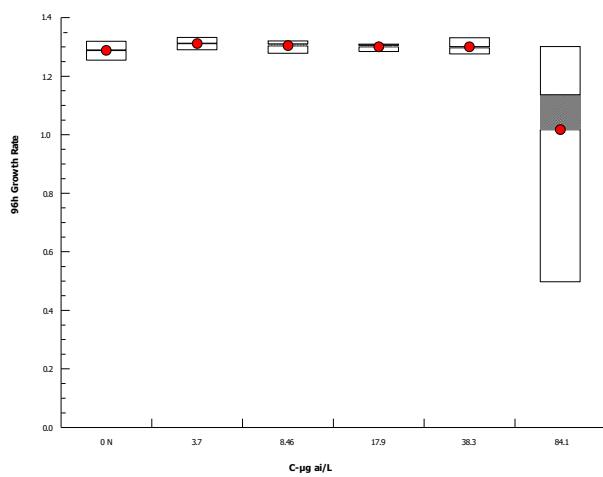


CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 6 of 6)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity								Wildlife International						
Analysis ID:		15-9459-2298	Endpoint:			96h Growth Rate			CETIS Version:		CETISv1.8.7			
Analyzed:		23 Mar-17 19:57	Analysis:			Nonparametric-Control vs Ord. Treatments			Official Results:		Yes			
Batch ID:	14-4880-3315	Test Type:			Algal Cell Growth (96-h)			Analyst:						
Start Date:	18 May-15	Protocol:			OCSPP 850.4500 Aquatic Plant (Algae)			Diluent:						
Ending Date:		Species:			Skeletonema costatum			Brine:						
Duration:	NA	Source:			Center for Culture of Marine Phytoplankton,			Age:						
Data Transform	Zeta	Alt Hyp	Trials	Seed				NOEL	LOEL	TOEL	TU			
Untransformed	NA	C > T	NA	NA				84.1	>84.1	NA	NA			
Jonckheere-Terpstra Step-Down Test														
Control	vs	C- μ g ai/L		Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)				
Negative Control		3.7		6	NA	-2	0.9636	Exact	Non-Significant Effect					
		8.46		23	NA	-2	0.9580	Exact	Non-Significant Effect					
		17.9		55	NA	-2	0.8818	Exact	Non-Significant Effect					
		38.3		-0.7433	1.645	3	-2	0.7713	Asymp	Non-Significant Effect				
		84.1		0.7879	1.645	3	-2	0.2154	Asymp	Non-Significant Effect				
ANOVA Table														
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision(α :5%)					
Between	0.2729224		0.05458447		5	2.891		0.0374	Significant Effect					
Error	0.4154215		0.0188828		22									
Total	0.6883439				27									
Distributional Tests														
Attribute	Test		Test Stat		Critical	P-Value		Decision(α :1%)						
Variances	Bartlett Equality of Variance		63.49		15.09	<0.0001		Unequal Variances						
Distribution	Shapiro-Wilk W Normality		0.5428		0.8975	<0.0001		Non-normal Distribution						
96h Growth Rate Summary														
C- μ g ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect			
0	Negative Control	8	1.288	1.271	1.304	1.289	1.254	1.319	0.006987	1.54%	0.0%			
3.7		4	1.311	1.282	1.34	1.312	1.289	1.332	0.008972	1.37%	-1.83%			
8.46		4	1.304	1.274	1.333	1.309	1.277	1.32	0.009295	1.43%	-1.26%			
17.9		4	1.3	1.282	1.318	1.304	1.283	1.309	0.005788	0.89%	-0.97%			
38.3		4	1.3	1.263	1.337	1.296	1.275	1.331	0.01159	1.78%	-0.95%			
84.1		4	1.017	0.4297	1.604	1.135	0.497	1.301	0.1845	36.29%	21.01%			

Graphics



CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 1 of 4)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity				Wildlife International
Analysis ID:	20-0380-1094	Endpoint:	96h AUC	CETIS Version: CETISv1.8.7
Analyzed:	23 Mar-17 19:57	Analysis:	Nonlinear Regression	Official Results: Yes
Batch ID:	14-4880-3315	Test Type:	Algal Cell Growth (96-h)	Analyst:
Start Date:	18 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent: Algal Culture Media
Ending Date:		Species:	Skeletonema costatum	Brine:
Duration:	NA	Source:	Center for Culture of Marine Phytoplankton,	Age:

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
65	-192.5	392.1	395.1	0.8040	Yes	0.9485	3.049	0.4343	Non-Significant Lack of Fit

Point Estimates

Level	µg ai/L	95% LCL	95% UCL
IC5	68.57	N/A	72.08
IC10	71.15	65.34	73.94
IC25	75.7	74.23	77.02
IC50	81.09	79.85	82.34

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	5579	127	5330	5827	43.92	<0.0001	Significant Parameter
C	0.102	0.02147	0.05989	0.1441	4.75	<0.0001	Significant Parameter
D	81.09	0.6228	79.87	82.31	130.2	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	43667430	43667430	1	112.8	<0.0001	Significant
Lack of Fit	1108704	369568.1	3	0.9485	0.4343	Non-Significant
Pure Error	8571865	389630.2	22			
Residual	9680569	387222.8	25			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	20.6	11.07	0.0010	Unequal Variances
	Mod Levene Equality of Variance	8.921	2.661	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9412	0.9264	0.1185	Normal Distribution
	Anderson-Darling A2 Normality	0.7872	2.492	0.0409	Non-normal Distribution

96h AUC Summary			Calculated Variate						
C-µg ai/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	5327	4855	5866	142.7	403.7	7.58%	0.0%
3.7		4	5619	5137	5885	175.9	351.8	6.26%	-5.5%
8.46		4	5518	5098	5797	150.5	301	5.46%	-3.59%
17.9		4	5902	5804	6037	49.1	98.19	1.66%	-10.8%
38.3		4	5780	5261	6133	186.9	373.8	6.47%	-8.51%
84.1		4	2010	318	3482	726.9	1454	72.33%	62.27%

CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 2 of 4)
Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity

Wildlife International

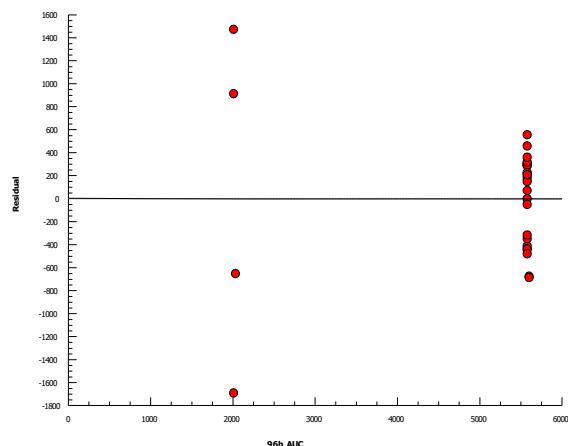
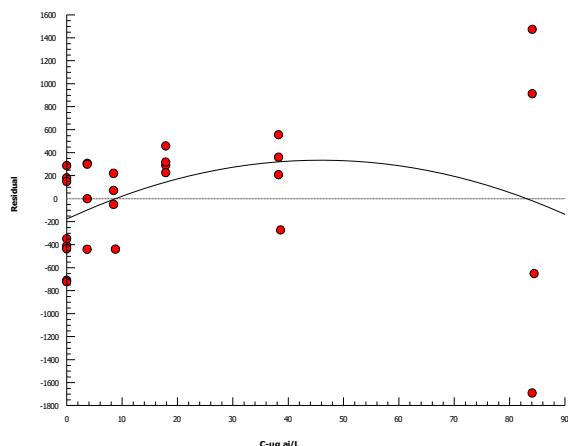
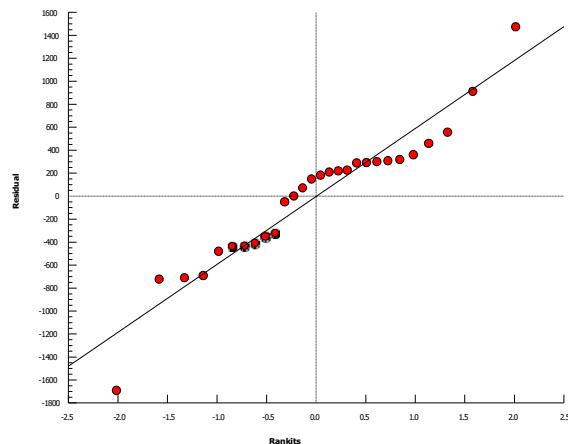
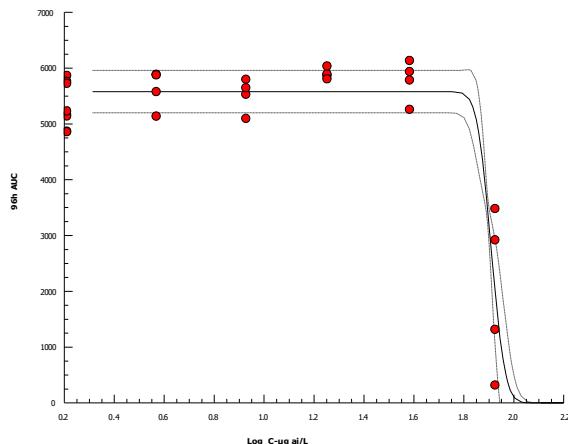
Analysis ID: 20-0380-1094
Analyzed: 23 Mar-17 19:57

Endpoint: 96h AUC
Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics

3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]



CETIS Analytical Report

Report Date: 24 Mar-17 09:43 (p 3 of 4)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity				Wildlife International
Analysis ID:	03-4874-4541	Endpoint:	96h Cell Density	CETIS Version: CETISv1.8.7
Analyzed:	23 Mar-17 19:57	Analysis:	Nonlinear Regression	Official Results: Yes
Batch ID:	14-4880-3315	Test Type:	Algal Cell Growth (96-h)	Analyst:
Start Date:	18 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent: Algal Culture Media
Ending Date:		Species:	Skeletonema costatum	Brine:
Duration:	NA	Source:	Center for Culture of Marine Phytoplankton,	Age:

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
32	-108.6	224.3	227.3	0.4384	Yes	0.2774	3.049	0.8411	Non-Significant Lack of Fit

Point Estimates

Level	µg ai/L	95% LCL	95% UCL
IC5	71.11	N/A	81.93
IC10	74.03	N/A	85.71
IC25	79.16	N/A	89.86
IC50	85.29	83.25	87.39

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	179.5	6.345	167	191.9	28.28	<0.0001	Significant Parameter
C	0.1105	0.09736	-0.08029	0.3014	1.135	0.2670	Non-Significant Parameter
D	85.29	1.057	83.22	87.36	80.67	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	22296.27	22296.27	1	23.08	<0.0001	Significant
Lack of Fit	880.4233	293.4745	3	0.2774	0.8411	Non-Significant
Pure Error	23274.76	1057.943	22			
Residual	24155.18	966.2072	25			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Mod Levene Equality of Variance	15.28	2.661	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8937	0.9264	0.0082	Non-normal Distribution
	Anderson-Darling A2 Normality	1.273	2.492	0.0023	Non-normal Distribution

96h Cell Density Summary

C-µg ai/L	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	171.9	149.6	194.2	4.783	13.53	7.87%	0.0%
3.7		4	188.9	172.5	205.2	6.826	13.65	7.23%	-9.89%
8.46		4	183.2	164.4	195.3	6.613	13.23	7.22%	-6.62%
17.9		4	180.4	168.3	186.7	4.101	8.201	4.55%	-4.93%
38.3		4	180.6	163.2	203.8	8.461	16.92	9.37%	-5.06%
84.1		4	98.83	6.3	180.8	40.67	81.34	82.31%	42.5%

CETIS Analytical Report

Report Date: 24 Mar-17 09:43 (p 4 of 4)
Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity

Wildlife International

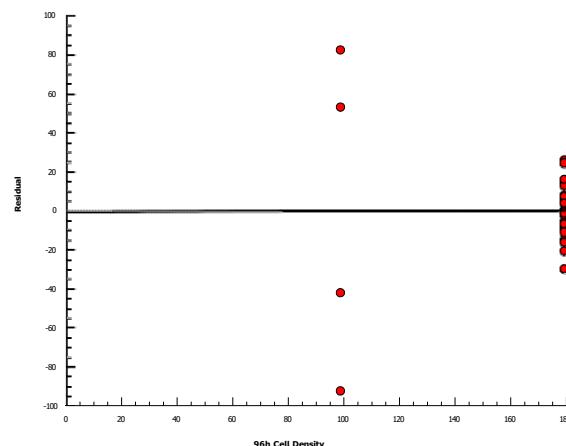
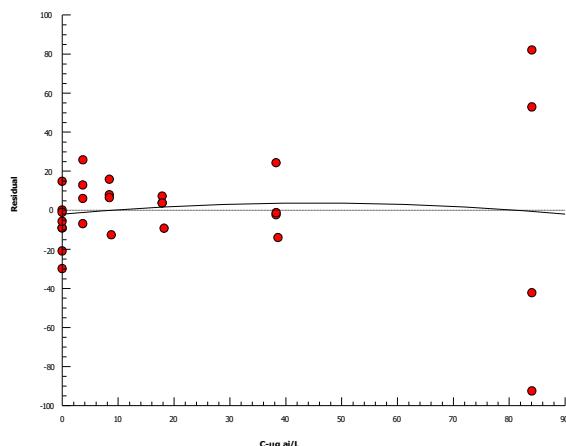
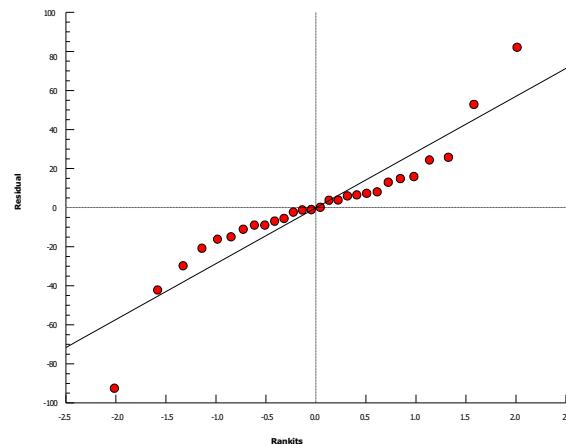
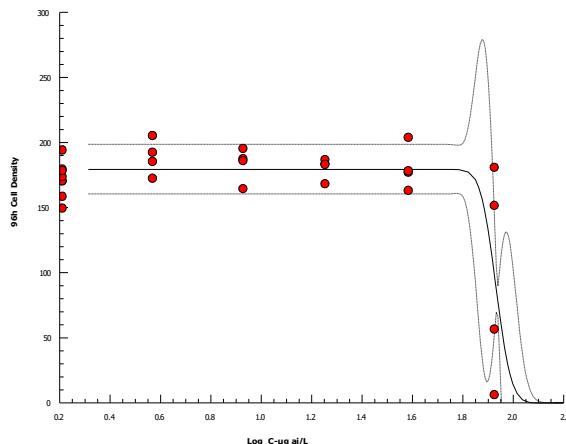
Analysis ID: 03-4874-4541
Analyzed: 23 Mar-17 19:57

Endpoint: 96h Cell Density
Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics

3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]



CETIS Summary Report

 Report Date: 24 Mar-17 09:43 (p 1 of 1)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity							Wildlife International
Batch ID:	14-4880-3315	Test Type: Algal Cell Growth (96-h)				Analyst:	
Start Date:	18 May-15	Protocol: OCSPP 850.4500 Aquatic Plant (Algae)				Diluent:	Algal Culture Media
Ending Date:		Species: Skeletonema costatum				Brine:	
Duration:	NA	Source: Center for Culture of Marine Phytoplankton, Age:					
Sample ID:	19-1062-9639	Code:	128994 49760108			Client:	CDM Smith - D. Worcester
Sample Date:	18 May-15	Material:	Dithiopyr			Project:	
Receive Date:		Source:	Dow AgroSciences				
Sample Age:	NA	Station:					

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-8417-1219	96h AUC	84.1	>84.1	NA	NA		Jonckheere-Terpstra Step-Down Test
12-1582-1418	96h AUC	38.3	84.1	56.75	13.0%		Mann-Whitney U Two-Sample Test
10-2424-2738	96h Cell Density	84.1	>84.1	NA	NA		Jonckheere-Terpstra Step-Down Test
17-7390-3534	96h Cell Density	84.1	>84.1	NA	21.0%		Mann-Whitney U Two-Sample Test
15-9459-2298	96h Growth Rate	84.1	>84.1	NA	NA		Jonckheere-Terpstra Step-Down Test
05-3046-2470	96h Growth Rate	84.1	>84.1	NA	11.8%		Mann-Whitney U Two-Sample Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg ai/L	95% LCL	95% UCL	TU	Method
20-0380-1094	96h AUC	IC5	68.57	N/A	72.08		Nonlinear Regression
		IC10	71.15	65.34	73.94		
		IC25	75.7	74.23	77.02		
		IC50	81.09	79.85	82.34		
03-4874-4541	96h Cell Density	IC5	71.11	N/A	81.93		Nonlinear Regression
		IC10	74.03	N/A	85.71		
		IC25	79.16	N/A	89.86		
		IC50	85.29	83.25	87.39		

96h AUC Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	5327	4989	5664	4855	5866	142.7	403.7	7.58%	0.0%
3.7		4	5619	5059	6179	5137	5885	175.9	351.8	6.26%	-5.5%
8.46		4	5518	5039	5997	5098	5797	150.5	301	5.46%	-3.59%
17.9		4	5902	5745	6058	5804	6037	49.09	98.19	1.66%	-10.8%
38.3		4	5780	5185	6374	5261	6133	186.9	373.8	6.47%	-8.51%
84.1		4	2010	-303.5	4323	318	3482	726.9	1454	72.33%	62.27%

96h Cell Density Summary

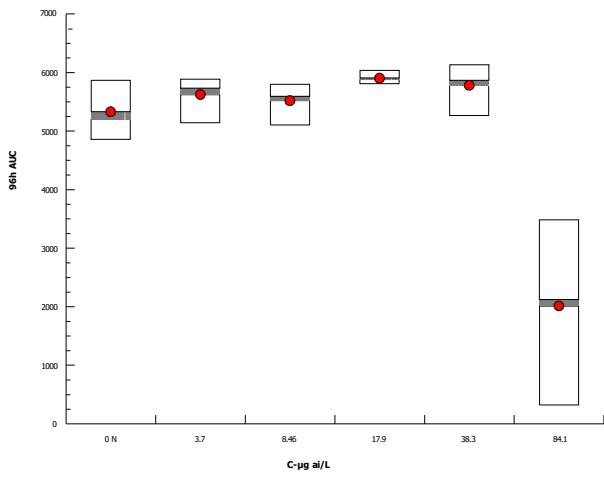
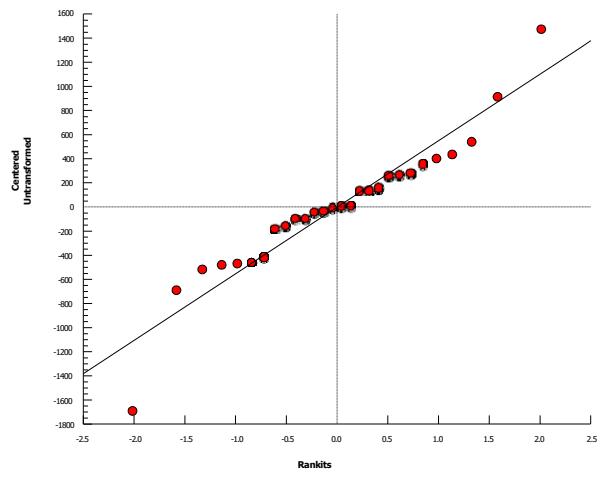
C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	171.9	160.6	183.2	149.6	194.2	4.783	13.53	7.87%	0.0%
3.7		4	188.9	167.2	210.6	172.5	205.2	6.826	13.65	7.23%	-9.89%
8.46		4	183.3	162.2	204.3	164.4	195.3	6.613	13.23	7.22%	-6.62%
17.9		4	180.4	167.3	193.4	168.3	186.7	4.101	8.201	4.55%	-4.93%
38.3		4	180.6	153.6	207.5	163.2	203.8	8.461	16.92	9.37%	-5.06%
84.1		4	98.83	-30.61	228.3	6.3	180.8	40.67	81.34	82.31%	42.5%

96h Growth Rate Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	1.288	1.271	1.304	1.254	1.319	0.006987	0.01976	1.54%	0.0%
3.7		4	1.311	1.282	1.34	1.289	1.332	0.008972	0.01794	1.37%	-1.83%
8.46		4	1.304	1.274	1.333	1.277	1.32	0.009295	0.01859	1.43%	-1.26%
17.9		4	1.3	1.282	1.318	1.283	1.309	0.005788	0.01158	0.89%	-0.97%
38.3		4	1.3	1.263	1.337	1.275	1.331	0.01159	0.02317	1.78%	-0.95%
84.1		4	1.017	0.4297	1.604	0.497	1.301	0.1845	0.3691	36.29%	21.01%

CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 1 of 6)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity								Wildlife International										
Analysis ID:		12-1582-1418	Endpoint:			96h AUC	CETIS Version:		CETISv1.8.7									
Analyzed:		23 Mar-17 19:57	Analysis:			Nonparametric-Two Sample	Official Results:		Yes									
Batch ID:	14-4880-3315	Test Type:			Algal Cell Growth (96-h)			Analyst:										
Start Date:	18 May-15	Protocol:			OCSPP 850.4500 Aquatic Plant (Algae)			Diluent:										
Ending Date:		Species:			Skeletonema costatum			Brine:										
Duration:	NA	Source:			Center for Culture of Marine Phytoplankton,			Age:										
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU									
Untransformed	NA	C > T	NA	NA	13.0%	38.3	84.1	56.75										
Mann-Whitney U Two-Sample Test																		
Control	vs	C-µg ai/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α :5%)									
Negative Control	3.7	9	NA	0	10	0.8929	Exact	Non-Significant Effect										
	8.46	13	NA	0	10	0.7152	Exact	Non-Significant Effect										
	17.9	1	NA	0	10	0.9980	Exact	Non-Significant Effect										
	38.3	4	NA	0	10	0.9859	Exact	Non-Significant Effect										
	84.1*	32	NA	0	10	0.0020	Exact	Significant Effect										
ANOVA Table																		
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α :5%)										
Between	44776130		8955227		5	22.98	<0.0001	Significant Effect										
Error	8571865		389630.2		22													
Total	53348000				27													
Distributional Tests																		
Attribute	Test		Test Stat	Critical	P-Value	Decision(α :1%)												
Variances	Bartlett Equality of Variance		20.6	15.09	0.0010	Unequal Variances												
Distribution	Shapiro-Wilk W Normality		0.9327	0.8975	0.0721	Normal Distribution												
96h AUC Summary																		
C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect							
0	Negative Control	8	5327	4989	5664	5198	4855	5866	142.7	7.58%	0.0%							
3.7		4	5619	5059	6179	5728	5137	5885	175.9	6.26%	-5.5%							
8.46		4	5518	5039	5997	5588	5098	5797	150.5	5.46%	-3.59%							
17.9		4	5902	5745	6058	5882	5804	6037	49.1	1.66%	-10.8%							
38.3		4	5780	5185	6374	5862	5261	6133	186.9	6.47%	-8.51%							
84.1		4	2010	-303.5	4323	2119	318	3482	726.9	72.33%	62.27%							
Graphics																		
																		

CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 2 of 6)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	11-8417-1219	Endpoint:	96h AUC	CETIS Version:	CETISv1.8.7
Analyzed:	23 Mar-17 19:57	Analysis:	Nonparametric-Control vs Ord. Treatments	Official Results:	Yes

Batch ID:	14-4880-3315	Test Type:	Algal Cell Growth (96-h)	Analyst:
Start Date:	18 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent: Algal Culture Media
Ending Date:		Species:	Skeletonema costatum	Brine:
Duration:	NA	Source:	Center for Culture of Marine Phytoplankton,	Age:

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	84.1	>84.1	NA	

Jonckheere-Terpstra Step-Down Test

Control	vs	C- μ g ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
Negative Control		3.7	9	NA	-2	0.9970	Exact	Non-Significant Effect	
		8.46	33	NA	-2	0.9970	Exact	Non-Significant Effect	
		17.9	38	NA	-2	0.9970	Exact	Non-Significant Effect	
		38.3	60	NA	-2	0.9970	Exact	Non-Significant Effect	
		84.1	156	NA	-2	0.5714	Exact	Non-Significant Effect	

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	44776130	8955227	5	22.98	<0.0001	Significant Effect
Error	8571865	389630.2	22			
Total	53348000		27			

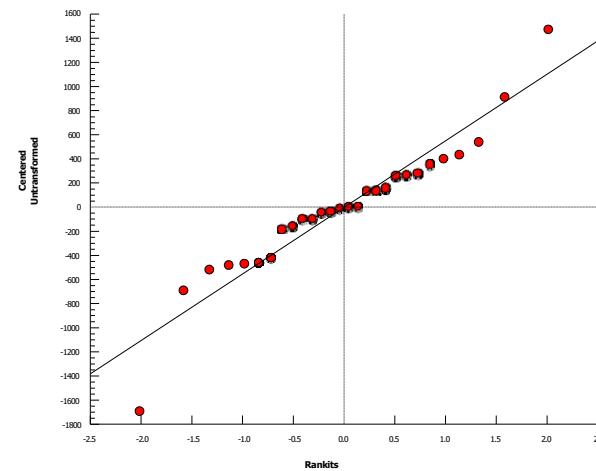
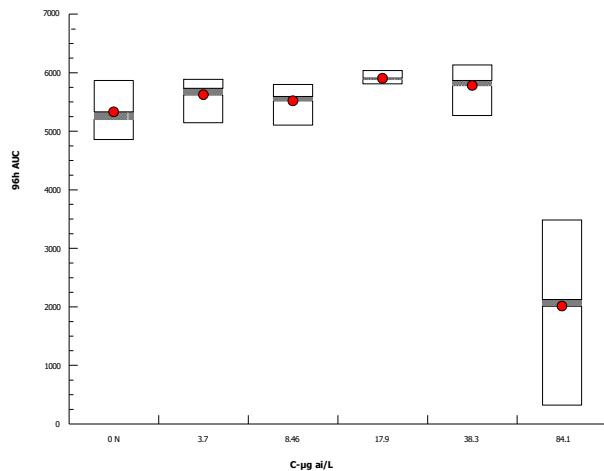
Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	20.6	15.09	0.0010	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9327	0.8975	0.0721	Normal Distribution

96h AUC Summary

C- μ g ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	5327	4989	5664	5198	4855	5866	142.7	7.58%	0.0%
3.7		4	5619	5059	5728	5137	5885	175.9	6.26%	-5.5%	
8.46		4	5518	5039	5997	5588	5098	5797	150.5	5.46%	-3.59%
17.9		4	5902	5745	6058	5882	5804	6037	49.1	1.66%	-10.8%
38.3		4	5780	5185	6374	5862	5261	6133	186.9	6.47%	-8.51%
84.1		4	2010	-303.5	4323	2119	318	3482	726.9	72.33%	62.27%

Graphics



CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 3 of 6)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	17-7390-3534	Endpoint:	96h Cell Density	CETIS Version:	CETISv1.8.7				
Analyzed:	23 Mar-17 19:57	Analysis:	Nonparametric-Two Sample	Official Results:	Yes				
Batch ID:	14-4880-3315	Test Type:	Algal Cell Growth (96-h)	Analyst:					
Start Date:	18 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media				
Ending Date:		Species:	Skeletonema costatum	Brine:					
Duration:	NA	Source:	Center for Culture of Marine Phytoplankton,	Age:					
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	21.0%	84.1	>84.1	NA	

Mann-Whitney U Two-Sample Test

Control	vs	C-µg ai/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		3.7	6	NA	0	10	0.9596	Exact	Non-Significant Effect
		8.46	8	NA	0	10	0.9172	Exact	Non-Significant Effect
		17.9	9	NA	0	10	0.8848	Exact	Non-Significant Effect
		38.3	12	NA	0	10	0.7576	Exact	Non-Significant Effect
		84.1	24	NA	0	10	0.1010	Exact	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	23176.69	4635.338	5	4.381	0.0064	Significant Effect
Error	23274.76	1057.943	22			
Total	46451.45		27			

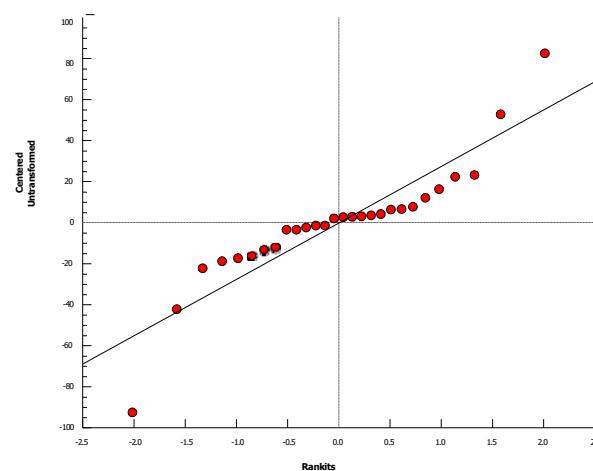
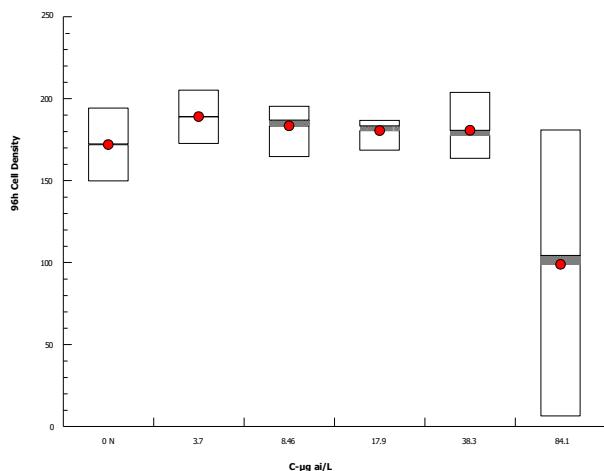
Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	26.46	15.09	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8641	0.8975	0.0018	Non-normal Distribution

96h Cell Density Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	171.9	160.6	183.2	172.1	149.6	194.2	4.783	7.87%	0.0%
3.7		4	188.9	167.2	210.6	188.9	172.5	205.2	6.826	7.23%	-9.89%
8.46		4	183.2	162.2	204.3	186.6	164.4	195.3	6.613	7.22%	-6.62%
17.9		4	180.4	167.3	193.4	183.2	168.3	186.7	4.101	4.55%	-4.93%
38.3		4	180.6	153.6	207.5	177.6	163.2	203.8	8.461	9.37%	-5.06%
84.1		4	98.83	-30.61	228.3	104.1	6.3	180.8	40.67	82.31%	42.5%

Graphics



CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 4 of 6)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	10-2424-2738	Endpoint:	96h Cell Density	CETIS Version:	CETISv1.8.7
Analyzed:	23 Mar-17 19:57	Analysis:	Nonparametric-Control vs Ord. Treatments	Official Results:	Yes
Batch ID:	14-4880-3315	Test Type:	Algal Cell Growth (96-h)	Analyst:	
Start Date:	18 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media
Ending Date:		Species:	Skeletonema costatum	Brine:	
Duration:	NA	Source:	Center for Culture of Marine Phytoplankton,	Age:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	84.1	>84.1	NA	

Jonckheere-Terpstra Step-Down Test

Control	vs	C-µg ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		3.7	6	NA	-2	0.9636	Exact	Non-Significant Effect	
		8.46	23	NA	-2	0.9580	Exact	Non-Significant Effect	
		17.9	55	NA	-2	0.8818	Exact	Non-Significant Effect	
		38.3	98	NA	-2	0.7688	Exact	Non-Significant Effect	
		84.1	180	NA	-2	0.2175	Exact	Non-Significant Effect	

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	23176.69	4635.338	5	4.381	0.0064	Significant Effect
Error	23274.76	1057.943	22			
Total	46451.45		27			

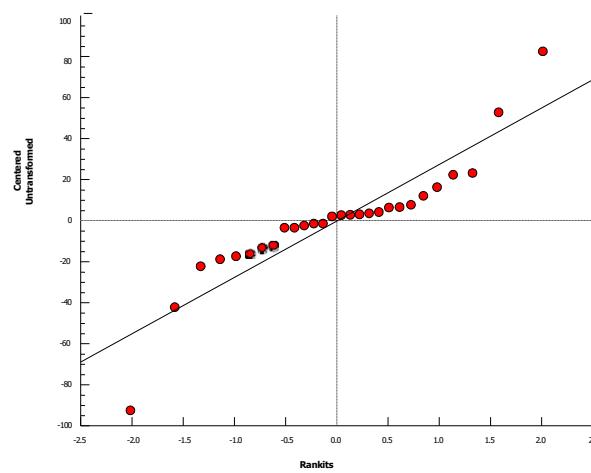
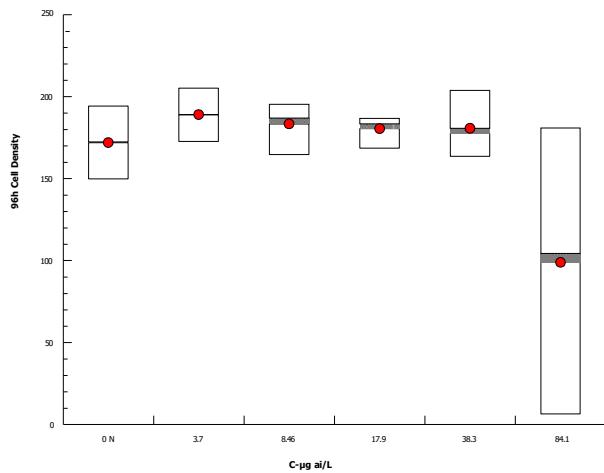
Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	26.46	15.09	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8641	0.8975	0.0018	Non-normal Distribution

96h Cell Density Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	171.9	160.6	183.2	172.1	149.6	194.2	4.783	7.87%	0.0%
3.7		4	188.9	167.2	210.6	188.9	172.5	205.2	6.826	7.23%	-9.89%
8.46		4	183.2	162.2	204.3	186.6	164.4	195.3	6.613	7.22%	-6.62%
17.9		4	180.4	167.3	193.4	183.2	168.3	186.7	4.101	4.55%	-4.93%
38.3		4	180.6	153.6	207.5	177.6	163.2	203.8	8.461	9.37%	-5.06%
84.1		4	98.83	-30.61	228.3	104.1	6.3	180.8	40.67	82.31%	42.5%

Graphics



OCSPP 850.4500 Algal Toxicity

Wildlife International

Analysis ID:	05-3046-2470	Endpoint:	96h Growth Rate	CETIS Version:	CETISv1.8.7
Analyzed:	23 Mar-17 19:57	Analysis:	Nonparametric-Two Sample	Official Results:	Yes
Batch ID:	14-4880-3315	Test Type:	Algal Cell Growth (96-h)	Analyst:	
Start Date:	18 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent:	Algal Culture Media
Ending Date:		Species:	Skeletonema costatum	Brine:	
Duration:	NA	Source:	Center for Culture of Marine Phytoplankton,	Age:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	11.8%	84.1	>84.1	NA	

Mann-Whitney U Two-Sample Test

Control	vs	C-µg ai/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Negative Control		3.7	6	NA	0	10	0.9596	Exact	Non-Significant Effect
		8.46	8	NA	0	10	0.9172	Exact	Non-Significant Effect
		17.9	9	NA	0	10	0.8869	Exact	Non-Significant Effect
		38.3	11.5	NA	1	10	0.7758	Exact	Non-Significant Effect
		84.1	24	NA	0	10	0.1010	Exact	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2729224	0.05458447	5	2.891	0.0374	Significant Effect
Error	0.4154215	0.0188828	22			
Total	0.6883439		27			

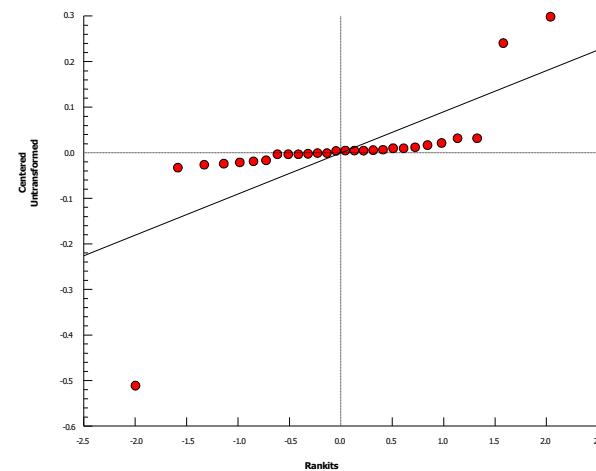
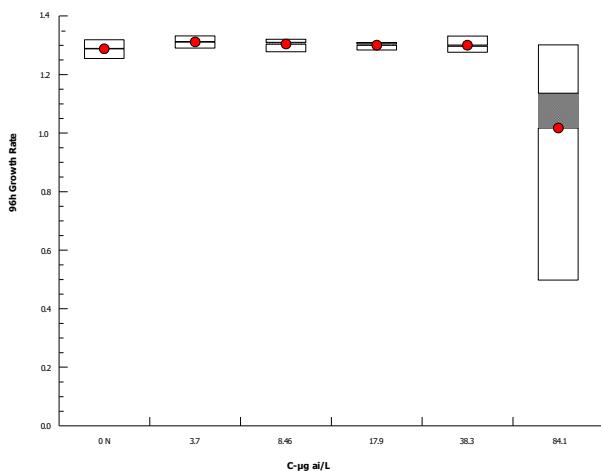
Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	63.49	15.09	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.5428	0.8975	<0.0001	Non-normal Distribution

96h Growth Rate Summary

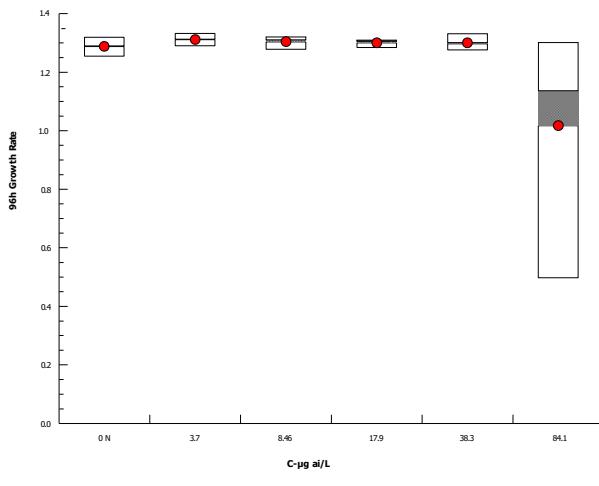
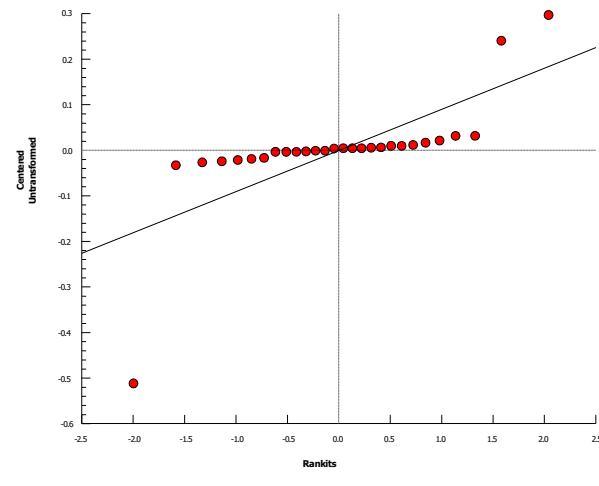
C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Negative Control	8	1.288	1.271	1.304	1.289	1.254	1.319	0.006987	1.54%	0.0%
3.7		4	1.311	1.282	1.34	1.312	1.289	1.332	0.008972	1.37%	-1.83%
8.46		4	1.304	1.274	1.333	1.309	1.277	1.32	0.009295	1.43%	-1.26%
17.9		4	1.3	1.282	1.318	1.304	1.283	1.309	0.005788	0.89%	-0.97%
38.3		4	1.3	1.263	1.337	1.296	1.275	1.331	0.01159	1.78%	-0.95%
84.1		4	1.017	0.4297	1.604	1.135	0.497	1.301	0.1845	36.29%	21.01%

Graphics



CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 6 of 6)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity							Wildlife International					
Analysis ID:		15-9459-2298	Endpoint:			96h Growth Rate	CETIS Version:		CETISv1.8.7			
Analyzed:		23 Mar-17 19:57	Analysis:			Nonparametric-Control vs Ord. Treatments	Official Results:		Yes			
Batch ID:	14-4880-3315	Test Type:			Algal Cell Growth (96-h)			Analyst:				
Start Date:	18 May-15	Protocol:			OCSPP 850.4500 Aquatic Plant (Algae)			Diluent:				
Ending Date:		Species:			Skeletonema costatum			Brine:				
Duration:	NA	Source:			Center for Culture of Marine Phytoplankton,			Age:				
Data Transform	Zeta	Alt Hyp	Trials	Seed			NOEL	LOEL	TOEL	TU		
Untransformed	NA	C > T	NA	NA			84.1	>84.1	NA			
Jonckheere-Terpstra Step-Down Test												
Control	vs	C- μ g ai/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)			
Negative Control		3.7	6	NA	-2	0.9636	Exact	Non-Significant Effect				
		8.46	23	NA	-2	0.9580	Exact	Non-Significant Effect				
		17.9	55	NA	-2	0.8818	Exact	Non-Significant Effect				
		38.3	-0.7433	1.645	3	-2	0.7713	Asymp	Non-Significant Effect			
		84.1	0.7879	1.645	3	-2	0.2154	Asymp	Non-Significant Effect			
ANOVA Table												
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision(α :5%)			
Between	0.2729224		0.05458447		5	2.891		0.0374	Significant Effect			
Error	0.4154215		0.0188828		22							
Total	0.6883439				27							
Distributional Tests												
Attribute	Test		Test Stat	Critical	P-Value	Decision(α :1%)						
Variances	Bartlett Equality of Variance		63.49	15.09	<0.0001	Unequal Variances						
Distribution	Shapiro-Wilk W Normality		0.5428	0.8975	<0.0001	Non-normal Distribution						
96h Growth Rate Summary												
C- μ g ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Negative Control	8	1.288	1.271	1.304	1.289	1.254	1.319	0.006987	1.54%	0.0%	
3.7		4	1.311	1.282	1.34	1.312	1.289	1.332	0.008972	1.37%	-1.83%	
8.46		4	1.304	1.274	1.333	1.309	1.277	1.32	0.009295	1.43%	-1.26%	
17.9		4	1.3	1.282	1.318	1.304	1.283	1.309	0.005788	0.89%	-0.97%	
38.3		4	1.3	1.263	1.337	1.296	1.275	1.331	0.01159	1.78%	-0.95%	
84.1		4	1.017	0.4297	1.604	1.135	0.497	1.301	0.1845	36.29%	21.01%	
Graphics												
												
												

CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 1 of 4)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity				Wildlife International
Analysis ID:	20-0380-1094	Endpoint:	96h AUC	CETIS Version: CETISv1.8.7
Analyzed:	23 Mar-17 19:57	Analysis:	Nonlinear Regression	Official Results: Yes
Batch ID:	14-4880-3315	Test Type:	Algal Cell Growth (96-h)	Analyst:
Start Date:	18 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent: Algal Culture Media
Ending Date:		Species:	Skeletonema costatum	Brine:
Duration:	NA	Source:	Center for Culture of Marine Phytoplankton,	Age:

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
65	-192.5	392.1	395.1	0.8040	Yes	0.9485	3.049	0.4343	Non-Significant Lack of Fit

Point Estimates

Level	µg ai/L	95% LCL	95% UCL
IC5	68.57	N/A	72.08
IC10	71.15	65.34	73.94
IC25	75.7	74.23	77.02
IC50	81.09	79.85	82.34

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	5579	127	5330	5827	43.92	<0.0001	Significant Parameter
C	0.102	0.02147	0.05989	0.1441	4.75	<0.0001	Significant Parameter
D	81.09	0.6228	79.87	82.31	130.2	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	43667430	43667430	1	112.8	<0.0001	Significant
Lack of Fit	1108704	369568.1	3	0.9485	0.4343	Non-Significant
Pure Error	8571865	389630.2	22			
Residual	9680569	387222.8	25			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Bartlett Equality of Variance	20.6	11.07	0.0010	Unequal Variances
	Mod Levene Equality of Variance	8.921	2.661	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9412	0.9264	0.1185	Normal Distribution
	Anderson-Darling A2 Normality	0.7872	2.492	0.0409	Non-normal Distribution

96h AUC Summary			Calculated Variate						
C-µg ai/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	5327	4855	5866	142.7	403.7	7.58%	0.0%
3.7		4	5619	5137	5885	175.9	351.8	6.26%	-5.5%
8.46		4	5518	5098	5797	150.5	301	5.46%	-3.59%
17.9		4	5902	5804	6037	49.1	98.19	1.66%	-10.8%
38.3		4	5780	5261	6133	186.9	373.8	6.47%	-8.51%
84.1		4	2010	318	3482	726.9	1454	72.33%	62.27%

CETIS Analytical Report

Report Date: 24 Mar-17 09:42 (p 2 of 4)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity

Wildlife International

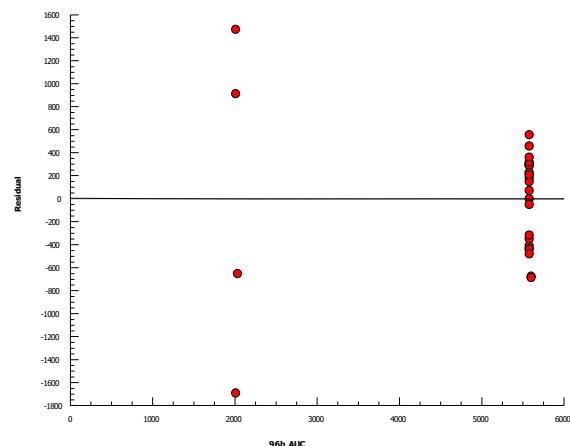
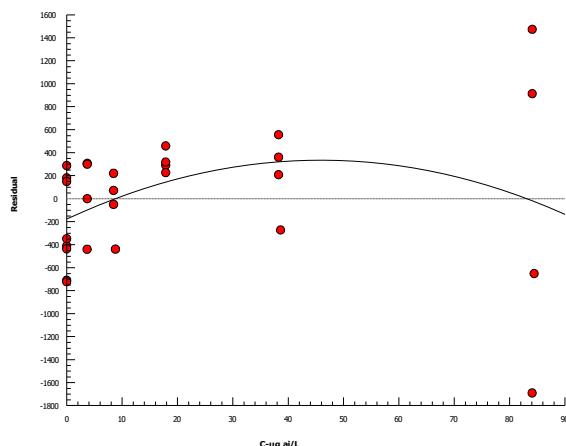
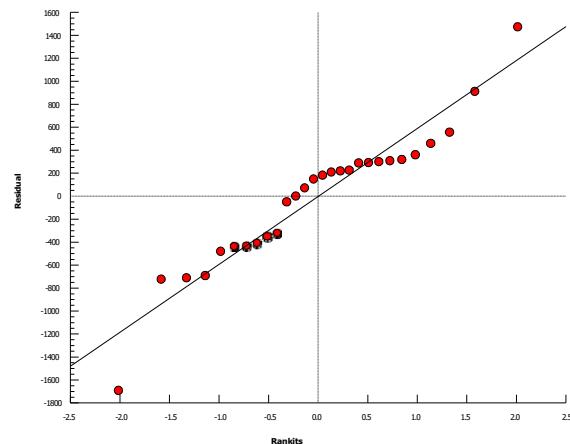
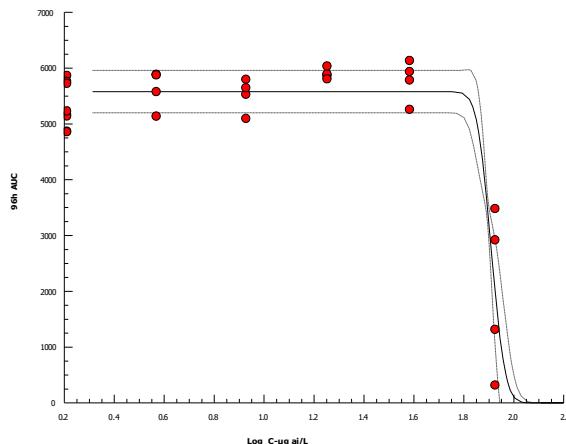
Analysis ID: 20-0380-1094
 Analyzed: 23 Mar-17 19:57

Endpoint: 96h AUC
 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
 Official Results: Yes

Graphics

3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]



CETIS Analytical Report

Report Date: 24 Mar-17 09:43 (p 3 of 4)
 Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity				Wildlife International
Analysis ID:	03-4874-4541	Endpoint:	96h Cell Density	CETIS Version: CETISv1.8.7
Analyzed:	23 Mar-17 19:57	Analysis:	Nonlinear Regression	Official Results: Yes
Batch ID:	14-4880-3315	Test Type:	Algal Cell Growth (96-h)	Analyst:
Start Date:	18 May-15	Protocol:	OCSPP 850.4500 Aquatic Plant (Algae)	Diluent: Algal Culture Media
Ending Date:		Species:	Skeletonema costatum	Brine:
Duration:	NA	Source:	Center for Culture of Marine Phytoplankton,	Age:

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
32	-108.6	224.3	227.3	0.4384	Yes	0.2774	3.049	0.8411	Non-Significant Lack of Fit

Point Estimates

Level	µg ai/L	95% LCL	95% UCL
IC5	71.11	N/A	81.93
IC10	74.03	N/A	85.71
IC25	79.16	N/A	89.86
IC50	85.29	83.25	87.39

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	179.5	6.345	167	191.9	28.28	<0.0001	Significant Parameter
C	0.1105	0.09736	-0.08029	0.3014	1.135	0.2670	Non-Significant Parameter
D	85.29	1.057	83.22	87.36	80.67	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	22296.27	22296.27	1	23.08	<0.0001	Significant
Lack of Fit	880.4233	293.4745	3	0.2774	0.8411	Non-Significant
Pure Error	23274.76	1057.943	22			
Residual	24155.18	966.2072	25			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Variances	Mod Levene Equality of Variance	15.28	2.661	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8937	0.9264	0.0082	Non-normal Distribution
	Anderson-Darling A2 Normality	1.273	2.492	0.0023	Non-normal Distribution

96h Cell Density Summary

C-µg ai/L	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	171.9	149.6	194.2	4.783	13.53	7.87%	0.0%
3.7		4	188.9	172.5	205.2	6.826	13.65	7.23%	-9.89%
8.46		4	183.2	164.4	195.3	6.613	13.23	7.22%	-6.62%
17.9		4	180.4	168.3	186.7	4.101	8.201	4.55%	-4.93%
38.3		4	180.6	163.2	203.8	8.461	16.92	9.37%	-5.06%
84.1		4	98.83	6.3	180.8	40.67	81.34	82.31%	42.5%

OCSPP 850.4500 Algal Toxicity

Wildlife International

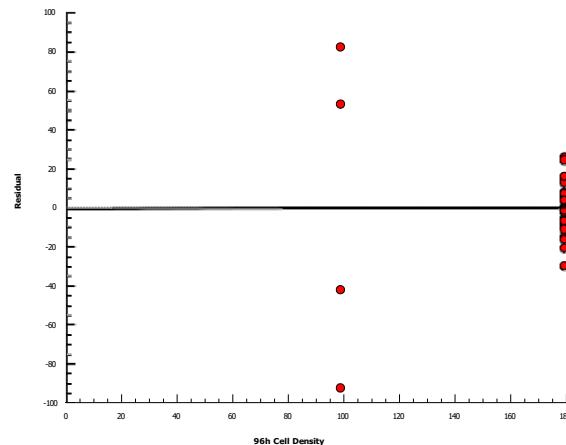
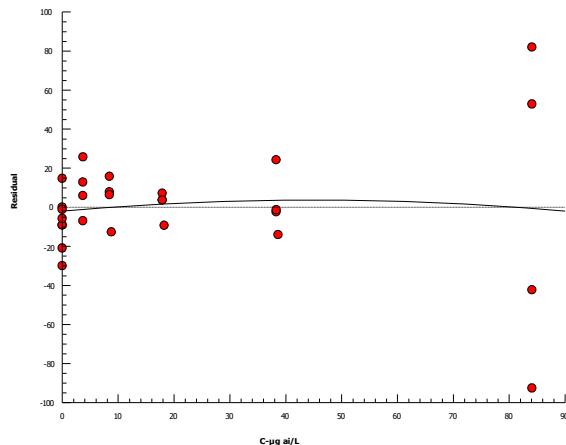
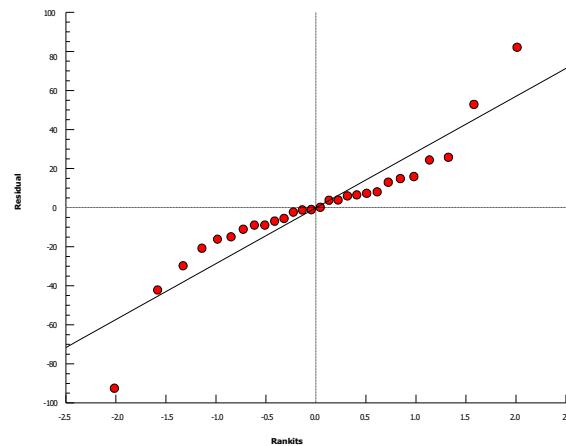
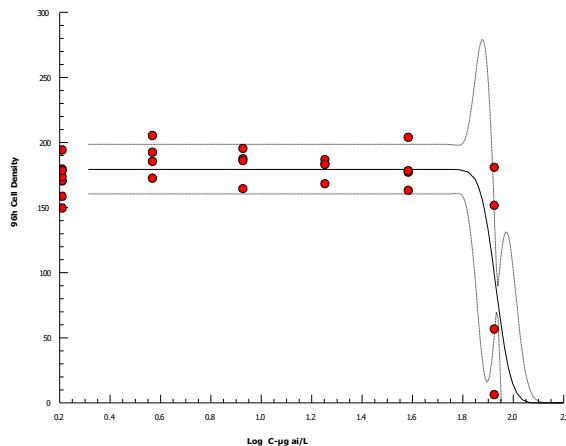
Analysis ID: 03-4874-4541
 Analyzed: 23 Mar-17 19:57

Endpoint: 96h Cell Density
 Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.7
 Official Results: Yes

Graphics

3P Cumulative Log-Normal EV [Y=A*(1- Φ(log(X/D)/C))]



CETIS Summary Report

Report Date: 24 Mar-17 09:43 (p 1 of 1)
Test Code: 128994 49760108 | 16-4825-2754

OCSPP 850.4500 Algal Toxicity							Wildlife International
Batch ID:	14-4880-3315	Test Type: Algal Cell Growth (96-h)				Analyst:	
Start Date:	18 May-15	Protocol: OCSPP 850.4500 Aquatic Plant (Algae)				Diluent:	Algal Culture Media
Ending Date:		Species: Skeletonema costatum				Brine:	
Duration:	NA	Source: Center for Culture of Marine Phytoplankton, Age:					
Sample ID:	19-1062-9639	Code:	128994 49760108			Client:	CDM Smith - D. Worcester
Sample Date:	18 May-15	Material:	Dithiopyr			Project:	
Receive Date:		Source:	Dow AgroSciences				
Sample Age:	NA	Station:					

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-8417-1219	96h AUC	84.1	>84.1	NA	NA		Jonckheere-Terpstra Step-Down Test
12-1582-1418	96h AUC	38.3	84.1	56.75	13.0%		Mann-Whitney U Two-Sample Test
10-2424-2738	96h Cell Density	84.1	>84.1	NA	NA		Jonckheere-Terpstra Step-Down Test
17-7390-3534	96h Cell Density	84.1	>84.1	NA	21.0%		Mann-Whitney U Two-Sample Test
15-9459-2298	96h Growth Rate	84.1	>84.1	NA	NA		Jonckheere-Terpstra Step-Down Test
05-3046-2470	96h Growth Rate	84.1	>84.1	NA	11.8%		Mann-Whitney U Two-Sample Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg ai/L	95% LCL	95% UCL	TU	Method
20-0380-1094	96h AUC	IC5	68.57	N/A	72.08		Nonlinear Regression
		IC10	71.15	65.34	73.94		
		IC25	75.7	74.23	77.02		
		IC50	81.09	79.85	82.34		
03-4874-4541	96h Cell Density	IC5	71.11	N/A	81.93		Nonlinear Regression
		IC10	74.03	N/A	85.71		
		IC25	79.16	N/A	89.86		
		IC50	85.29	83.25	87.39		

96h AUC Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	5327	4989	5664	4855	5866	142.7	403.7	7.58%	0.0%
3.7		4	5619	5059	6179	5137	5885	175.9	351.8	6.26%	-5.5%
8.46		4	5518	5039	5997	5098	5797	150.5	301	5.46%	-3.59%
17.9		4	5902	5745	6058	5804	6037	49.09	98.19	1.66%	-10.8%
38.3		4	5780	5185	6374	5261	6133	186.9	373.8	6.47%	-8.51%
84.1		4	2010	-303.5	4323	318	3482	726.9	1454	72.33%	62.27%

96h Cell Density Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	171.9	160.6	183.2	149.6	194.2	4.783	13.53	7.87%	0.0%
3.7		4	188.9	167.2	210.6	172.5	205.2	6.826	13.65	7.23%	-9.89%
8.46		4	183.3	162.2	204.3	164.4	195.3	6.613	13.23	7.22%	-6.62%
17.9		4	180.4	167.3	193.4	168.3	186.7	4.101	8.201	4.55%	-4.93%
38.3		4	180.6	153.6	207.5	163.2	203.8	8.461	16.92	9.37%	-5.06%
84.1		4	98.83	-30.61	228.3	6.3	180.8	40.67	81.34	82.31%	42.5%

96h Growth Rate Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	8	1.288	1.271	1.304	1.254	1.319	0.006987	0.01976	1.54%	0.0%
3.7		4	1.311	1.282	1.34	1.289	1.332	0.008972	0.01794	1.37%	-1.83%
8.46		4	1.304	1.274	1.333	1.277	1.32	0.009295	0.01859	1.43%	-1.26%
17.9		4	1.3	1.282	1.318	1.283	1.309	0.005788	0.01158	0.89%	-0.97%
38.3		4	1.3	1.263	1.337	1.275	1.331	0.01159	0.02317	1.78%	-0.95%
84.1		4	1.017	0.4297	1.604	0.497	1.301	0.1845	0.3691	36.29%	21.01%